Application No.: 10/717,677

Response dated: March 25, 2008

Reply to Office Action dated: September 27, 2007

## Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

## **Listing of Claims:**

- (withdrawn-previously amended) A method of culturing human embryonic stem 1. (ES) cells with reduced differentiation comprising:
- a) growing the human ES cells in culture on a flexible solid porous matrix without conditioned media and in the absence of fibroblast feeder cells; and
- applying an effective amount of periodic strain on the flexible matrix to stretch **b**) the matrix and the human ES cells thereon, such that the human ES cells proliferate and exhibit reduced differentiation relative to human ES cells not subjected to periodic strain.
  - 2. -3. canceled.
- 4. (withdrawn) The method of Claim 1 wherein the cell differentiation is eliminated.
- 5. (withdrawn) The method of Claim 1 wherein the cells are grown on Matrigel<sup>TM</sup> using BioFlex® untreated culture plates.
- (withdrawn) The method of Claim 1 wherein the cells are grown without the 6. presence of cross-species biological material.
  - (withdrawn) The method of Claim 1 wherein the flexible matrix is Matrigel<sup>TM</sup>. 7.
  - 8. (withdrawn) The method of Claim 1 wherein the strain is mechanically produced.

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- 9. (withdrawn) The method of Claim 1 wherein the flexible matrix is stretched using vacuum pressure.
- (withdrawn) The method of Claim 1 wherein the strain exerted on the flexible 10. matrix is at least about 5%.
- (withdrawn) The method of Claim 1 wherein the flexible matrix undergoes at 11. least about 6 stretches per minute.
- (withdrawn) The method of Claim 1 wherein the mechanical strain is from 12. oscillatory stretching of the flexible matrix surface.
- 13. (currently amended) A cell culture composition comprising: proliferating human embryonic stem (ES) cells in culture in an unconditioned culture medium on a flexible solid porous matrix without conditioned media or fibroblast feeder cells; a flexible solid porous matrix, wherein the cells are on the matrix; and

in an apparatus for applying an effective amount of configured to apply periodic strain on to the flexible matrix to stretch the matrix and the human ES cells thereon, such that wherein more of the human ES cells proliferate and exhibit reduced differentiation relative to in the culture medium are undifferentiated than in an otherwise comparable cell culture composition comprising an apparatus not configured to apply periodic strain to the matrix and the human ES cells-not subjected to periodic strain.

## 14.-15. canceled.

(currently amended) The culture composition of Claim 13 wherein the cell 16. differentiation is eliminated substantially all of the human ES cells in the culture are undifferentiated.

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(currently amended) The culture composition of Claim 13 wherein the cells are 17. grown on matrix comprises Matrigel<sup>TM</sup> using and the apparatus comprises a BioFlex® untreated culture plates plate.

- 18. (currently amended) The culture composition of Claim 13 wherein the cells are grown without the presence of culture medium is free of cross-species biological material.
- 19. (currently amended) The culture composition of Claim 13 wherein the flexible solid porous matrix is comprises Matrigel<sup>TM</sup>.
- (currently amended) The culture composition of Claim 13 wherein the apparatus 20. is configured to apply mechanical strain is mechanically produced to the matrix and the human ES cells.
- (currently amended) The culture composition of Claim 13 wherein the flexible 21. matrix is stretched using apparatus is configured to apply vacuum pressure to the matrix and the human ES cells.
- 22. (currently amended) The culture composition of Claim [[13]] 20 wherein the mechanical strain is from comprises oscillatory stretching of the flexible matrix surface.
- 23. (currently amended) The culture composition of Claim 13 wherein the apparatus is configured to exert at least about 5% strain exerted on the flexible matrix is at least about 5%.
- (currently amended) The culture composition of Claim 13 wherein the flexible 24. apparatus is configured to stretch the matrix undergoes at least about 6 stretches times per minute.

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- 25. (withdrawn-previously presented) A method of culturing human embryonic stem (ES) cells with reduced differentiation comprising:
- a) growing the human ES cells in culture on a flexible solid porous matrix without conditioned media and in the absence of fibroblast feeder cells; and
- b) applying an effective amount of periodic strain on the human ES cells, such that the human ES cells proliferate and exhibit reduced differentiation relative to human ES cells not subjected to periodic strain.
- 26. (currently amended) A cell <u>The</u> culture composition comprising:

  undifferentiated human stem cells in culture without conditioned media or fibroblast

  feeder cells of Claim 13, wherein the stem <u>human ES</u> cells are defined <u>characterized</u> by the

  positive expression of Oct4 and SSEA-4 cell surface markers;

a flexible solid porous matrix, wherein the cells are on the matrix; and
an apparatus for applying an effective amount of periodic strain on the flexible matrix to
stretch the matrix and the undifferentiated human stem cells thereon, such that the human stem
cells proliferate and exhibit reduced differentiation relative to undifferentiated human stem cells
not subjected to periodic strain.

27. (presently amended) The method <u>culture composition</u> of Claim 26, wherein the <u>undifferentiated</u> human stem <u>ES</u> cells are also immuno-positive for <del>presence of</del> alkaline phosphatase.